AD_____

Award Number:
W81XWH-10-1-0106

TITLE:
The Oncogenic Palmitoyl-Protein Network in Prostate Cancer

PRINCIPAL INVESTIGATOR:
Michael R. Freeman, PhD

CONTRACTING ORGANIZATION:
Children's Hospital
Boston, MA 02115

REPORT DATE:
March 2011

TYPE OF REPORT:
Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

X Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
31 March 2011	Annual	01 MAR 2010 - 28 FEB 2011

4. TITLE AND SUBTITLE
The Oncogenic Palmitoyl-Protein Network in Prostate Cancer

*xxxxxxxxxxxxxx
5a. CONTRACT NUMBER
PC093459

5b. GRANT NUMBER
W81XWH-10-1-0106

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Michael R. Freeman, PhD
michael.freeman@childrens.harvard.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Children's Hospital
Enders 1161
300 Longwood Avenue
Boston, MA 02115-5737

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research
and Materiel Command
Ft. Detrick, MD 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)
USAMRAA

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

X Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Molecular studies of cancer cells and tissues have revealed that the basis for malignant tumor formation, growth, and spread is the disruption of normal patterns of gene and protein expression. Although this concept is fundamental to our understanding of cancer, genes and proteins also interact with a wide range of fat (lipid) molecules in a complex metabolic network. Studies of this lipid network have lagged considerably behind those of genetic mutations and signal transduction cascades. However, evidence from laboratory research as well as studies of human patients indicates that lipid metabolism plays a major role in clinical progression of prostate cancer (PCa) and other solid tumors. For example, population studies indicate that obesity is a risk factor for castrate-resistant PCa, and drugs that lower circulating cholesterol appear to reduce the risk of disease progression.

Cell culture, animal experiments, and studies of human tumors have identified an enzyme, fatty acid synthase (FASN), as a biochemically relevant protein in PCa. FASN has been described as a "metabolic oncogene," which operates by an unknown mechanism to promote tumor growth. We know that FASN is the source of a class of lipids called long-chain fatty acids. Most of the long-chain fatty acids synthesized by FASN are used to produce cell membranes and to modify proteins through a process called palmitoylation (where the fatty acid palmitate, or a closely related fatty acid, is affixed to proteins). Studies from our laboratory and others indicate that these lipid products of FASN are related to mechanisms by which cholesterol can promote tumor growth and spread.

In this project, we will use a sophisticated method of protein analysis, involving mass spectrometry-based proteomics, to identify lipid-modified proteins downstream from FASN. We argue that these protein targets are potentially novel, and significant, in that they may regulate tumor cell growth and other behaviors relevant to metastatic dissemination, such as cell motility and the production of membrane particles capable of modifying the tumor microenvironment. Our approach will for the first time unveil this FASN signaling network, helping us to understand how FASN works as an oncoprotein and how this molecule or its synthetic products might be targeted using novel approaches. We will also test, using a model of human tumor growth and spread from the prostate, whether this FASN network is vulnerable to a diet and drug intervention that will employ a Food and Drug Administration-approved cholesterol-reducing agent (ezetimibe). These studies will provide new mechanistic data on important mediators of PCa progression within the complex web of lipid metabolism, will identify new protein and lipid targets for intervention and clinical assessment, and may produce important preclinical data on the potential chemopreventive or therapeutic value of ezetimibe.

15. SUBJECT TERMS
castrate-resistant prostate cancer, palmitoylation, signal transduction, S-acylation

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			Michael R. Freeman
Unclassified	Unclassified	Unclassified	Unlimited	7	**19b. TELEPHONE NUMBER** *(include area code)* 617-919-2644

Table of Contents

Page

The Oncogenic Palmitoyl-Protein Network in Prostate Cancer
Grant number: PC093459
Progress report for 1 MAR 2010 - 28 FEB 2011

INTRODUCTION

This project seeks to expand recent observations from our laboratory and others suggesting (1) that fatty acid synthase- (FASN-) dependent palmitoylation (S-acylation) and cholesterol-sensitive signaling mechanisms intersect during progression of prostate cancer (PCa) to castration resistant disease; (2) that FASN lies upstream from a largely unknown palmitoylated protein signaling network; and (3) that this network might be sensitive to pharmacologic targeting of cholesterol using the cholesterol-lowering drug, ezetimibe. Our hypothesis is that PCa progression is dependent on a palmitoyl-protein network regulated by FASN. We predict that the activity of this network can be suppressed by pharmacologic reduction of circulating cholesterol level.

The specific aims of the project are:

Aim 1: Identify critical palmitoyl-proteins in the FASN subnetwork. Test their functional roles.
Aim 2: Determine whether the FASN-palmitoyl network can be suppressed in vivo by cholesterol reduction.

BODY

Our primary objective in this study is to apply a novel method of palmitoyl-proteome analysis (PalmPISC) developed by our laboratory (Yang *et al.*, Mol Cell Proteomics 9: 54-70, 2010) in combination with other mass spectrometry and cell biological approaches, to identify critical nodes and network relationships in the FASN-dependent palmitoyl-protein network in PCa cells. In the past funding year, we have made substantial progress on experiments proposed in Aim 1.

Identification of > 200 novel palmitoyl proteins. This year, as a result of support from this funding mechanism, we made the surprising discovery that a large population of palmitoyl proteins are localized in nuclei-associated membrane structures of human cells. Proteomic analysis of palmitoyl proteins enriched from the nuclear membrane fraction led to the discovery *of over **200** novel palmitoyl protein candidates*. This is one of the largest collection of newly-identified palmitoyl-proteins ever assembled in any mammalian system. Using acyl-biotinyl exchange (ABE) chemistry coupled with western blotting, we verified the palmitoylation of eukaryotic initiation factor 3H (eIF3H) and ribosomal protein S9 (RPS9). In addition, we applied the PalmPISC method to analyze palmitoyl proteins enriched from human platelets. Among the 215 identified palmitoyl protein candidates in this cell background, 103 proteins have not been reported previously. By using ^{3}H-palmitate labeling, we verified palmitoylation of TREM-like transcript-1, a protein specifically expressed in platelets and megakaryocytes.

Palmitoyl proteins regulated by EGF. We applied triplex SILAC-based quantitative proteomics to identify, in an unbiased manner, palmitoyl proteins and membrane proteins regulated by EGF stimulation of DU145 PCa cells for 0, 5, or 40 min. Intriguingly, the palmitoylation level of a variety of translation factors, including many ribosomal proteins and several eukaryotic initiation factors, was found to be about 1.3~1.5-fold increased after 5 or 40 min EGF stimulation (Fig. 1). The rapid increase of the palmitoylation level of those translation factors significantly increased the level of membrane-associated translation machinery in the cell (data not shown). Given that membrane-bound ribosomes usually produce proteins that are associated with the plasma membrane or are secreted, the upregulation of membrane-localized translation machinery may stimulate the synthesis of certain plasma membrane

proteins or secreted proteins, some of which may be involved in cancer progression. In addition, it has been reported that mammalian target of rapamycin complex 2 (mTORC2), the S473-Akt kinase, is activated by association with the ribosome (Zinzalla *et al.*, Cell 144: 757, 2011). It is likely that the enzymes regulating the palmitoylation of ribosomal proteins are the missing link between EGF and ribosome-mTORC2. This pathway has been implicated in progression of PCa to castrate-resistant disease. We are currently challenging this hypothesis by performing functional assays. Notably, these findings are consistent with our original hypothesis that protein palmitoylation plays a major role in oncogenic signaling mechanisms in PCa.

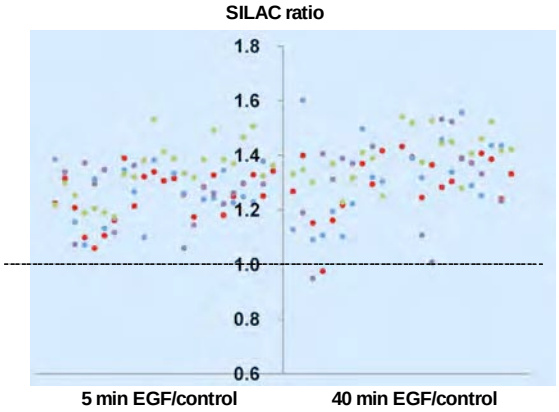

Figure 1. Quantitative palmitoyl-proteomics analysis suggests that the palmitoylation level of many translation factors increases upon EGF stimulation of PCa cells. Dots indicate translation factors and colors represent different membrane domains. The vast majority of palmitoylated proteins was not significantly changed after 5 or 40 min EGF stimulation (data not shown).

REPORTABLE OUTCOMES

Yang, W., Di Vizio, D., Kirchner, M., Steen, H., and Freeman, M.R. (2010) Proteome-scale characterization of human S-acylated proteins in lipid raft-enriched and non-raft membranes. Molecular and Cellular Proteomics . 9:54-70. PMID: 19801377.

Pelton, K., Di Vizio, D., Insabato, L., Schaffner, C.P., Freeman, M.R., and Solomon, K.R. (2010) Ezetimibe reduces enlarged prostate in an animal model of benign prostatic hyperplasia. Journal of Urology 184:1555-1559. PMID: 20728125.

Wu, X., Gao, H., Ke, W., Hager, M., Xiao, S., Freeman, M.R., and Zhu, Z. (2011) VentX trans-activates p53 and p16ink4a to regulate cellular senescence. Journal of Biological Chemistry 286:12693-12701. PMID: 21325273.

KEY RESEARCH ACCOMPLISHMENTS

--Identification of over 200 likely palmitoylated human proteins, including 103 *novel* palmitoyl-protein candidates.
--Demonstration by quantitative proteomics that EGFR activation of human PCa cells results in extensive changes in the palmitoyl-proteome, with increases in palmitoylation of many proteins associated with the translational machinery of the cell. These findings appear to link EGFR-responsive protein palmitoylation with the mTORC2-Akt pathway.

CONCLUSIONS

We have made substantial progress in year 1 in reconstruction of the palmitoyl-protein signal transduction network in PCa cells. We are using PCa cells, platelets and other cell types to make inferences about possible tissue- and/or cell-specific functions of these proteins and we are performing functional assessments as outlined in our DoD proposal. The dataset we have assembled to date suggests the surprising observations that the palmitoyl-proteome in PCa cells is intimately linked to (1) regulation of specialized nuclear functions and (2) protein translation. These studies are ongoing.

Personnel Receiving Salary from W81XWH-10-1-0106

1. Michael R. Freeman, PhD
2. Martin Hager, PhD
3. Matteo Morello, PhD
4. Hanno Steen, PhD
5. Wei Yang, PhD